Journal of Bacteriology 70 #4 (October 1955) 485-486.

P61

A SIMPLIFIED DEVICE FOR CONTINUOUS GROWTH OF MICROORGANISMS[1]

BORIS ROTMAN

Institute for Enzyme Research and the Department of Genetics, University of Wisconsin, Madison, Wisconsin

Received for publication April 18, 1955

One of the frequent problems of the microbiologist is to obtain microorganisms grown under the same chemical and physical conditions. A number of methods have been published providing satisfactory means to maintain a culture in continuous growth (Monod, Ann. inst. Pasteur, **79**, 390, 1950; Novick and Szilard, Cold Spring Harbor Symposia Quant. Biol., **16**, 337, 1951; Kubitschek, J. Bacteriol., **67**, 254, 1954) and it has been the common assumption that the microorganisms are physiologically identical under the conditions described. Evidence in favor of this assumption will be published elsewhere.

Although the apparatus so far described in the literature are suitable for specialized problems, they lack the simplicity desirable for routine work in microbiology. This communication presents a modification of the chemostat of Novick and Szilard offering a reduction in cost and ease of operation and maintaining at the same time a reasonable level of accuracy.

Figure 1 shows the complete apparatus with the exception of the air supply and the constant temperature bath which are standard equipment in any laboratory. The apparatus is made of three pieces connected with glass joints and held together by springs.[2] Each part is autoclaved separately and then, after the medium in the Erlenmeyer flasks has equilibrated with room temperature, the apparatus is assembled upside down, inverted and suspended from a ring stand over the constant temperature bath so as to have the culture submerged completely. The method is based on the delivery of nutrient solution at a constant rate into a constant volume of culture. The nutrient solution contains limiting amounts of an essential metabolite. In our case the regular flow of nutrient is achieved by the constant level in the middle receptacle maintained by the content of the 1-L Erlenmeyer flask. The flow rate can be regulated by means of the stopcock which has a fine groove at each end of the plug to permit fine adjustment. Teflon stopcocks can be also used

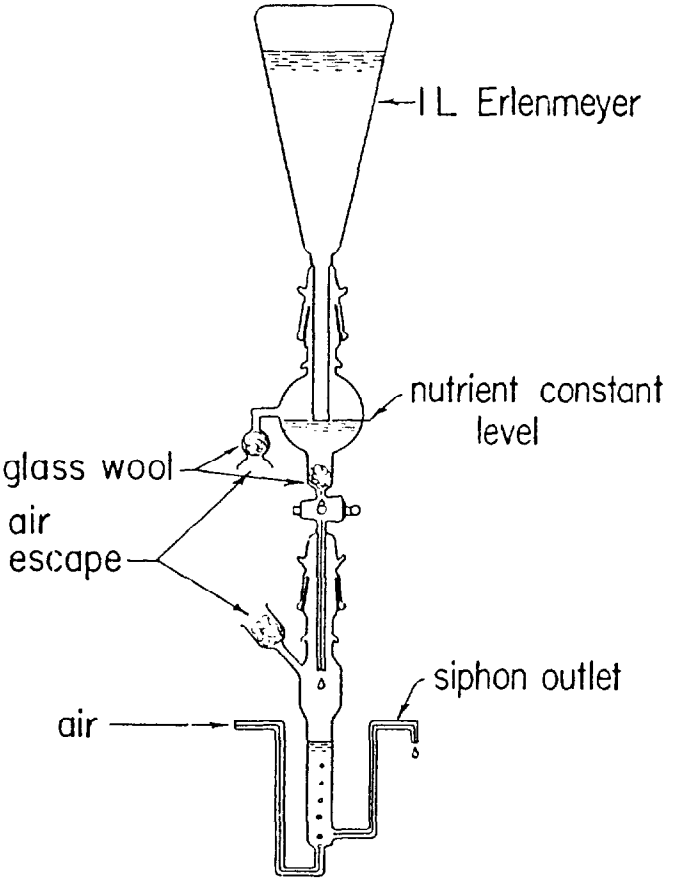

Figure 1

[1] Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council. Suggestions from Dr. R. E. Parks on the construction of the apparatus are gratefully acknowledged.

[2] The complete apparatus may be obtained from the Erway Glass Blowing Co., Oregon, Wisconsin.

when grease is to be avoided (space for expansion during autoclaving must be left because Teflon has a different temperature coefficient from glass). The air escape in the growth tubes serves also as an inoculation port. Closing the air escape causes a rise in air pressure, forcing the content of the growth tube through the siphon outlet. Increase in atmospheric pressure does not affect the apparatus. Decreases in atmospheric pressure when operating at low flow rates are accommodated by the large surface area of the middle compartment. Extreme decreases in atmospheric pressure would cause the nutrient to overflow around the air escape which can then be sterilized by a flame. The piece of glass wool on top of the stopcock prevents solid particles from clotting the feeding tube, but it is advisable to filter the nutrient solution prior to sterilization.

The apparatus as described here has been used in this laboratory for a period of 5 months with flow rates ranging from 0.7 to 15 ml per hour. Fifteen determinations of the time necessary to deliver 2 ml gave an average of 34.3 min with a standard deviation of 1.2 min and a spread from 33.5 to 35.0 min using 3.5 ml per hr flow rate. The average flow rate during an 8-day period was 1.98 ml/hr with a spread of 1.8 to 2.2 ml/hr with a standard deviation of 0.23 ml/hr

For routine work it is advisable not to use selective media and to start a fresh culture every 10 days if selection of mutants is to be avoided. In our laboratory, using *Escherichia coli* with genetic markers, we have not detected selection over periods ranging from 10 to 20 days using an average generation time of 5 hours.